29.6.2015 Interlab measurement

MONDAY, 6/29

Did 1,3 % agarose gel: weighted 0,65 g agarose and mixed with 50 ml 1x TAE buffer. Added a drop of ETBR.

AHD6 and AHD7 from the kit:

Diluted plasmids for 10ul H20 and incubated in room temperature for 10 minutes. Liquid turned to red-colored. Mixed gently with pipette and transfered liquid into 0,5ml eppendorf (find names, companies and robustness). The markins of the DNA-tubes were AHD6 M and AHD7 M.

Trans: Inc at 37C started at 11:50 and end at 12:55, the cells were 30 min in room temp. after the step. Plate inc. started at 13.50

Started transformation for D1, D2, D3 into TOP10, Control w. plasmid, Control w/o plasmid, and Control w. insert in plasmid:

Added 50 ul of ice cold competent cells into pre-chilled 2ml tube**.** Added 2 ul of resuspended DNA to the same tube, and mixed with pipette gently. Liquid was transparent, some moisture on the tube walls. The cells were incubated on ice for 30 mins. The tubes were put in a water bath (42C) for 60s. After the heat shock the cells were on ice incubation for 5 minutes. Incubated the cells at 37C for 1 hr 5 mins with shaking (230 RPM). At this point there seemed to be growth in tubes because the liquid turned to be little bleary.

Petri dishers were labeled for each transformation and control with the volumes of 50 ul and 150 ul. However, controls without plasmids were labeled on normal LB-plate and chloramphenicol-plate, both of which volumes were 100 ul. Thus, 11 of plates were with chloramphenicol and one with normal LB. The incubation of plates (37C, 230 RPM) started at 14:50 and ended

Tube information:

Microcentrifuge/eppendorf tube with lid, polypropane, 2ml (Lid membrane with piercing area)
RCF max. (at 20C for 20 min): 20000g
Lid-diameter: 8,5 mm
Thickness of lid mebrane: 0,3 mm
Outer diameter: 10,8 mm
Height: 41,15 mm
Manufacturer: Brand GAMBH + CO KG
Cat.No 780540

Microcentrifuge/eppendorf tubes with lid, 1,5ml, PP (Lid membrane with piercing area)
RCF max: 30000g
Lid-diameter: 8,3 mm
Thickness lid membrane: 0,4 mm
Outer diameter: 10,75 mm
Height:40,8 mm
Manufacturer: Brand GAMBH + CO KG
Cat.No 780550

Microcentrifuge tubes 0,5 ml with lid, PP

RCF max: 30000g
Outer diameter: 8 mm
Height: 32 mm

OR

RCF max: 10000g
Outer diameter: 10 mm
Height: 30 mm

Manufacturer: Brand GAMBH + CO KG

Cat.No 780550

30.6.2015 Measurement study

TUESDAY, 6/30

Took the plates from incubator at 10:00, and checked correct colonies with UV-light. Only D2 (50ul and 150ul) and control AHD6 (+ control) showed green clear fluorescent light in couple colonies. Other plates (D1,D3 and negative controls) showed normal dim glow with all colonies but didn't give any fluorescent signal. The plates were also overgrowth with numerous colonies which counting was too hard. The plates were put back in the 37C at 11.00 and will be taken out afternoon, so that more GFP-protein could be generated. Overnight liquid culture were made from D2 and -control without any plasmids. The colonies for that were gathered from 50ul plates. Incubation of o/n cultures started at 16.00.

Possible reasons why there wasn't GFP-expressing colonies in D1 and D3 (- Controls shouldn't have them):

-The O/N cultivation was too fast (20 h) so the expression couldn't happen

-The restriction of some parts and ligation wasn't succesful. However, because D2 worked, we can assume that parts AHD2,AHD4 and AHD5 were restricted and ligated correctly, so there might be problems with parts AHD1 and AHD3.

1.7.2015 Measurement analysis

WEDNESDAY, 7/1

Labeled yesterday's D2 on the chloramphenicol plate and started overnight plate culture at 11.00 from yesterday's liquid culture.

Started ligation making the following mixtures:

D1 (Device 1) assembly:
Ligation mix:
5,1µl restricted AHD1
7,2µl restricted AHD4
5,0µl restricted AHD5
2,0µl 10x T4 DNA Ligase
0,2µl T4 DNA Ligase

D2 (Device 2) assembly:
Ligation mix:
5,1µl restricted AHD2
5µl restricted AHD4
5µl restricted AHD5
2,0µl 10x T4 DNA Ligase
0,2µl T4 DNA Ligase

D3 (Device 3) assembly:
Ligation mix:
5,1µl restricted AHD3
7,0µl restricted AHD4
5,0µl restricted AHD5
2,0µl 10x T4 DNA Ligase
0,2µl T4 DNA Ligase

Only the D1 had optimal amounts of inserts and backbone. With the D2 only 5ul of ADH4 was added, and for D3 7,0ul of ADH4, because the restricted DNA was running out. Incubated the samples 10 min at 22C and inactivated the enzyme at 65C, 10 mins.

Started the first incubation of transformation of f AHD6, AHD7, D1, D2, D3 and control without plasmid (wP) at 12.38. Added 2 ml DNA to each transformation andd 2 ml water to VP. After adding 200 ul SOC-media to each transformation incubated the tubes 1h in 37C, 230 rpm. Incubation started at 13.32.
Plated all the transformants to chloramphenicol plates (25 ul each) and WP (25 ul) to antibiotic-free LB plate. Left the plates to 37 C incubator at 15.10

o/n cultures of AHD6 & AHD7

2.7.2015 Measurement analysis

THURSDAY, 7/2

Only the ligation of D2 was succesful, it has 4 colonies with green fluorescence emission when observing under UV-light and rest of them was normal colonies without GFP. Positive control had also all colonies with GFP and the negative controls had normal colonies as expected. However, the plate for first D2 (made on 26.6) didn't show any emissions, and the colonies had grown together and the plate plate was fully covered by them. The other plates contained normal colonies without GFP, so there might be ligations with inserts and old backbones, because the colonies had antibiotic resistanse. These plates without GFP were moved to incubator (37C) at 11.00 for waiting protein expression and taken out during afternoon at 15.20. The overnight liquid culture of succesful D2 with incubation tube was made again and the incubation started at 12.00.

Possible reasons for failure:

-Ligation protocol didn't work even when the other ligations had right DNA-amounts of inserts and backbones. The ratio of inserts and backbones might be too small (1:1).
-Because D2 worked again but D1 and D3 not, AHD2, AHD4 and AHD5 can be expected to be properly restricted. The restrictions of AHD1 and AHD3 were treated with gel electrophoresis which gel-mix samples contained 5ul of DNA and 1 ul 6x loading dye. The agarose gel (1,3 %) were made of 0,65 g agarose to 50 ml TAEE-buffer. Started gel run at 10.34, 100V for 1,5 hr.

Started working with minipreparations of plasmids from the overnight cultures of AHD6 and AHD7.
The overnight cultures were transfered into 2 ml eppendorf-tubes and the cells were pelleted with the eppendorf-centrifuge (Eppendorf AG Inc. Hamburg, Centrifuge 5418, 12000 rpm, 2 mins). Added 250 ul Resuspension buffer A1 and vortexed the mixture until the cells resuspended. Added 250 ul Lysis buffer A2 (Sodium hydroxide solution 0,5-2,0 %) and inverted the tubes 5 times, which transferred liquid to blue-colored. Incubated 2 mins at room temperature for cell lysis, and added 350 ul Neutralization Buffer A3 (Guanidine hydrochloride 36-50 %) and inverted the tubes until the liquid was colorless. Buffer produced mucous sediment in the liquid, which seemed to be dead cell's remains. Centrifuged for 3 mins at 12000 RPM to pellet precipitate. Loaded clear supernatant onto the NucleioSpin Plasmid EasyPure Columns which were put into collection tubes (2 mL, Machenery-Nagel Inc., plastic information not available). Centrifuged for 30 s at the speed of 8000 rpm and discarded the flow-through. Added 450 ul Wash Buffer AQ to the spin column, and centrifuged for 1 min at the speed of 12000 rpm. Repeated the last step one more time in order to get the spin columndry. Added 50 ul Elution Buffer AE (5 mM Tris/HVl, pH 8,5) onto the middle of the column and incubated for 1 min. Centrifuged the mixture for 1 min at the speed of 12000 rpm, and the flowthrough was transparent. The purified liquid was measurede by NanoDrop.

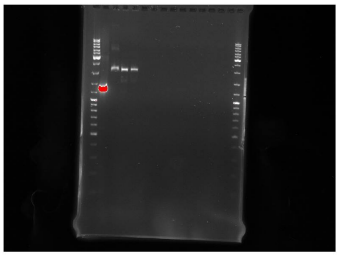
The following results were obtained from NanoDrop Lite (Thermo Scientific Inc.), shown in Table 1.

Table1

Sample	DNA (ng/µl)	Absorbance (A260/A280)
AHD6	68,8	1,84
AHD7	64,2	1,90

The sizes of AHD1, AHD2 and AHD3 restriction DNA can be seen in Figure 1, where the samples have been injected in 3rd, 4th and 5th wells and ladder in 1st well.

11664886_10206185214021897_573663997_o.jpg



Fiqure 1. Gel purification for AHD1, AHD2 and AHD3

The insert length for promoters are 35 bp, which means that these can't be detected properly with gel electrophoresis. However, the lengths of backbone/insert combination for AHD1, AHD2 and AHD3 are 2105 bp and without insert 2070 bp, and the gel samples have settled to the same location. In the gel image, the sample AHD2 seems to be slightly lower than AHD1 and AHD3. Therefore, the insert has been removed by restriction from AHD2 but not from AHD1 and AHD3. The earlier problems with ligations may be resulted from the unsufficient restrictions of AHD1 and AHD3.

3.7.2015 Interlab Measurement

TUESDAY, 8/4

Did GeneJet Plasmid Purify minipreparation for the yesteray's overnight culture of succesful D2. The following results were obtained:

NanoDrop results for D2 (Table 1).

Table1

Sample	DNA (ng/µl)	A260/A280
D2 (J23106 + I13504 +pSB1C3)	169,0	1,84